

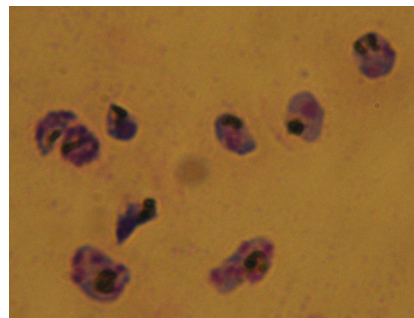
Identification of malaria parasite-infected red blood cell surface aptamers by inertial microfluidic SELEX (I-SELEX)

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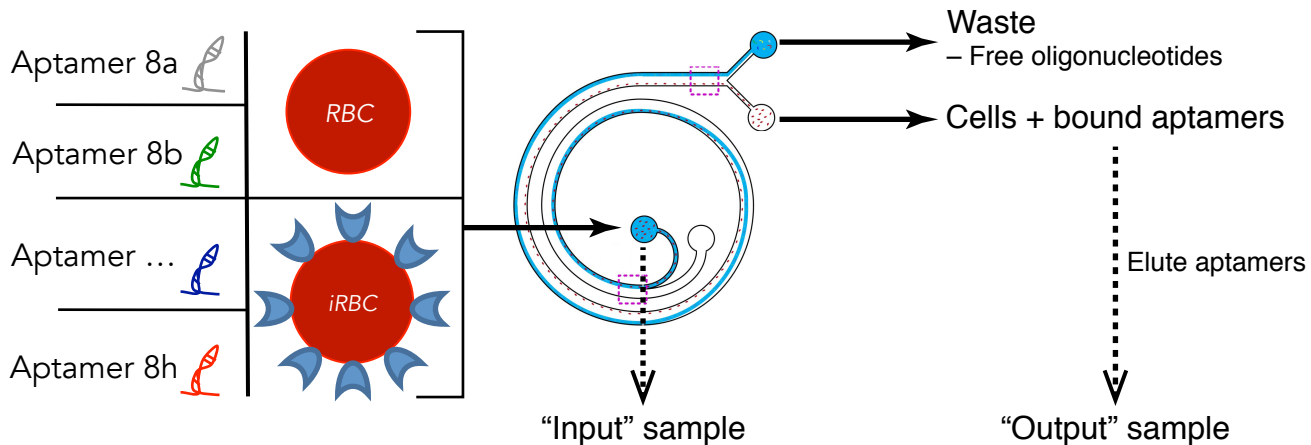
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MACS-enriched iRBCs



Supplementary Figure S1. A schematic of the aptamer enrichment assay. A single aptamer species is incubated with a target cell type (CS2 iRBCs, protease-treated iRBCs, DC-J iRBCs) or a negative control cell type (uninfected, mock-cultured RBCs). A small fraction of the reaction mixture is measured by qPCR to verify the “input” amount of aptamer. After incubation, the reaction mixture is passed once through the inertial microfluidic device, partitioning unbound aptamers from cells. Aptamers bound to the cell surface are eluted and quantified by qPCR as the “output” amount of aptamer. As measured by qPCR, all input samples have identical Crossing Points (Cp) and output samples are dependent on reaction conditions. The difference between input and output Cps (ΔCp) is used to calculate aptamer recovery. Aptamer recovery between target and control cell types ($\Delta\Delta\text{Cp}$) is used to establish statistical significance of aptamer enrichment.

Supplementary Table S1. Sequences recovered from de novo I-SELEX on tRBC targets.

Clone ID	K_d (nM)	Sequence of variable region
Cluster I		
3-25		GUUACUG AUCUUCUGCAGCGCGAAUCACAUUGAUGAAGCCGGAUCGACG
3-30	A	GUUACUG CGCUCUUACGAGGUAAUCUUAUGUUGGCAUUCGUAGUACU
5-1	A	GUUACUG AGCUCUUGUGUGUACAGUUGAGAAUCACAACGAUCCUCUG
5-2	A	GUUACUG GGUUCCCCUCCCAACCGCAUCGUAUGCCGGCAACCGA
5-5	2	A GUUACUG AGUUCGGGGAGGGGUAGAGCUCUACCGCCCAAUAGCGA
5-8		GUUACUG GGUUCCGCACAAAUGAGAUUUUUGUUUUUUAUCUGCCUCA
5-10		GUUACUG AGAGCCUUGACCCUCUGGAGCCACGACGUCGUGAAUUGAGG
5-11		GUUACUG ACACUCCCGUUGGUGCGAAGCACCAGUAACGACAGACUCAG
5-12	2	GUUACUG AGCUCAGUCGGGUGACGCGUCACCCUCUAGGAGAGACUCUGU
5-14	A	GUUACUG AUGUCCCGGAACUGGUGGGCAUUGGCAGACAUUGCAGCAGG
5-15	A	GUUACUG CGCUCUUAUCUGGCUCAUUGUGCCCGCAUCGAGGACAUG
5-17		GUUACUG AACUCUCGCGGUGCGCCACGUUGAUUGCAGUCGACUUCAG
Cluster II		
Toggle-25	40	GAACAAAGCUGAAGUAC UUAACCAAG AUCAUCCCGA
3-4		UGAUGUAAGACUGUUAUUGUGUGUUA UUGCCCAAG UUUGGUCUGUAUUG
3-10		UGGUGACUGGGUGAGAAUAGCAU UUGCCCAAG UCAUUCGAGUCUACAAC
3-19 (2)	> 1000	CUGCCUGAGACUGCACUUUUCGCUCC UUCCCCAAG UCUIUUUGGAGUU
5-6		CGAAUGCCGAAAUAAGAGCGUG UUCUCCAAG CCCGACUCGUACGGCUCGA
Cluster III*		
3-1		UCAAAUUAGUGCGCGAGGACUAUUAGGACGUCAGUACAUAUAUCAGAA
3-2		CGAAAAACAGUAUACGAGCAUCCUUCGUAUCCACCUUGAAAUAUAAAC
3-5		UAAGUUGCAGUAUUGGAGUCCUGAAAUGGACGUACGGUCCUUAU
3-6		UAGUCAUUGGUAAAAACAGUCUCCAAUCGAAUAUUACCGGUUAUGUUU
3-7 (4)	> 1000	CAACGGAUACACAGCAAGCGAAUCUUAUGUCCUUCGGGUCUAAAGGUCGCU
3-8		UCCAUUUCUUCGAAACGAUGAGCAC
3-9	4	ACGAACGUACAAGUUUAUGAAGAACUGACCCUUAUUGUCAUCCACACUCA
3-11		ACUUAAGAAUCCGACCGGGGUGACAAUUAUCCUUCUUGUUUUGUUAAC
3-12	200	GAUCAAUUUAUAUAUCCGACUCGAGAUUAUAUAUAUUGUAGUGG
3-13		ACAGGUUUUAAGUUUUCGUUUCGUAUGGAUUUUAACAAUCUAGAUC
3-14		AUAAGCUGAUUUUUCAGAACAUUUUAUAUACAGUCACACAGGCUCGAG
3-15		CCAUUUUAAGGAUACCGCUGCCCUACCCUUGCGGUUCAGCCGACCUUU
3-16		GGUUUUAAUUUAGUACUUCUUAUAGUUUUAUAGCCUUGAACAUUGGGA
3-17		CUUUGCCUUAUAUAUAUAGGUAACAUAAGUAGUGUAACGUUUGGUA
3-18		UACCGUUUAAGAAUGAGUUCAGAGUAAAAGUUUAUGUCCUGCAUGUCUAG
3-24		UGGGAGCCGAUAUAAGCAAUGUAUCACUUGCGCAUUGAAGUCGGACU
3-26		CAUUUGCGCGAAUCAGGCUUGCUUGUUAACACUGCAACGGUUUUAUCG
3-27		CCGCGACUUGCACUGUACCGCGUACCUUGCCUAUUGUUCUGUAAAAGAA
3-28		AAGGUUGAUGGCGUUGAUUCUUAUGUCAUUAUUCGUUUGCUUUAAGACGU
3-29		AUGAUUGAUUAUCAUGAAAAUGGCGUGGUGUAACACUACCCUGAUUUG
5-7		UUGCACCAAGGUUCGAAAUAUGAGCGGGCAGCGUCGAAUGCCGUAAU

Supplementary Table S2. Sequences recovered from de novo I-SELEX on malaria-parasite infected red blood cell targets. Clones are grouped by the stringency of the selection from which they were derived: Selection 1 (increased stringency for Rounds 6-8) and Selection 2 (consistent stringency across all 8 rounds). Tested sequences are shown in bold alongside their enrichment values ($\Delta\Delta C_p$) and the statistical significance (P value) of enrichment over scrambled negative control sequences (scr8.1-1 and scr8.2-1).

Clone ID	Copy #	$\Delta\Delta C_p$	P value	Sequence of variable region
Selection 1				
8.1-1	4	3.115	0.0002	CAACGGATCACACGCAAGCGAATCTATGTCCTTCGGGTCTAAGGGTCGCT
8.1-2	2	3.405	0.027	GTCAGAGTATGGGTACTGCAAGCTGCATGTGTTGCTCCGCTTAGACCCCTA
8.1-3	1	1.476	> 0.1	TCCCGCGCCATGGCGCCGGGAGCATGCGAGTCGGGCCAATTCGCC
8.1-4	1	1.965	> 0.1	GTCCGGTAGCGAGAGGACTTGGCAGCTTTGGTGAGCATAAGCTGTAGAC
8.1-5	1	3.18	0.0378	GTAAGCGGACGATGGTGCGTGGGCGAATTGATCAGTACTTACTGTGCGAA
8.1-6	1	-	-	GTGGAATCAACCCCGACTTAAAAACCTAACCGGATTCGGTTTGGCGAA
8.1-7	1	-	-	GGCATCGATTGTATTACTCATGTCTCCGAGTACTCCGAATGGCCGGCGT
8.1-8	1	-	-	GTGAGTGTGTTGACAATCGGAGCTAAGATGATGGCCGTGGTCAACGGA
8.1-9	1	-	-	TGTAGAGATTTCTGGAGCGTCAAAAGTAGGATGCCAAGATCGGTTGGA
8.1-10	1	-	-	GAGTGGTATCGGATCGGACGAATAGATTGCCCGTTAGGATCGTACCTGA
8.1-11	1	-	-	TTTGGAGCGATATGCGATATATCGATTGTAGATGAAGTCATGCAGTGGAA
8.1-12	1	-	-	GACAAAGATCCTAAAACATGCGCGTAACCGGACAGTATTGTAGCTTTGTG
Selection 2				
8.2-1	3	2.631	0.0159	CCAGGGTAAGTTAGTGTAGGACTTGTCTACATAGGATCGAATCGGTGG
8.2-2	3	3.388	0.0243	CTCGGATACTTAATAATAGAATTGGCCAGACGCATGCATATCCGCCAG
8.2-3	1	3.125	0.0131	GGCCTATCTGGGAGGAAAGCAGCCCAATTCGAACAGGTATGTGTGATCTG
8.2-4	1	-	-	AGGAAACTAATGTTGATACAACCTGGGTACAATTAGTATCCCGGTGATCT
8.2-5	1	-	-	CTTTTGTGTGATCGTTTATCCTAATGGTTTATCCGAGCTGGGATA
8.2-6	1	-	-	GGAGTATCAATCGGCACAGGTATGAGCAATCAGTTATGCACGTTTCCG
8.2-7	1	-	-	GGGATTGGGTGTCAGTCTGTAGAGTAGGTTACAGTATGAGCTTCA
8.2-8	1	-	-	AATTGGTCCGAATGACGGAGTTTTCGTCTAGATGCTAGGAAATGTTGCC
8.2-9	1	-	-	GTATAGGGTACTGTTCTGCTGCATTGGCCAGGTCATATAAGTCAGTTCT
8.2-10	1	-	-	TGGTGGTCGTCTCAAAAACCGCTATTGCGATGGATAGGCGTATTGCTC
8.2-11	1	-	-	GCAATGATTTGCATATCCTCTGAGCTGTGATCACCGCTAACATAACAGTC
8.2-12	1	-	-	TGACTTAAATGCCGAAGCGTTGACGTGATGGAGGATTTGCCGAACATTC
8.2-13	1	-	-	TGAAAGCACTACATTTATTGGTGATGAAAGTGCCTGATGCTGTTCCGC
Scrambled Sequences				
scr-8.1-1	n/a	1.243	null	ATGTCTCCAGCTCTTGTGGCGAACCAAGCGGGTTGACCAGAATGCATCA
scr-8.2-1	n/a	1.302	null	GAATGGTGTATATGTTGACAGTCAAGCGTCTCAGGTGCGGTAAGTCAGT

Supplementary Table S3. List of primers used in this work.

CMB49	CCGCTCGAGTAATACGACTCACTATAGGGAGAGAGGAA
CMB50	CTTCAGCTTTGTTCCCCATCCCTCTTCCCTCTCCCTATAGTGAGTC
CMB51	GATGGGGAACAAAGCTGAAGTACTTACCCAAGATCATCCCGAACGA
CMB56	TCGACCTCTGGGTTATGTCGTTTCGGGATGATCTTGGG
CMB77	GGGAGAGAGGAAGAGGGGATGGG
CMB94	TCGACCTCTGGGTTATGTCGTTTC
CMB95	CCGCTCGAGTAATACGACTCACTATAGGGAGACAAGAT
CMB96	GCAGCGTTCCCTCGATGGCCTTGATCTTGTCTCCCTATAGTGAGTCG
CMB97	CATCGAGGAACGCTGCCTACACACATGGACATAAACAAGAGCCGA)
CMB98	CCATTTTCTCGCCCTCTTCGGCTCTTGTTTATGTCCATG
CMB104	TTCTCGCCCTCTTCGGCTCTTGTTTA
CMB106	GAGACAAGATCAAGGCCATCGAGGAA
CMB107	CACTATAGGGAGCTCAGAATAAACGCTCAAACGAACGTACAAGTTATGAGAACTG
CMB108	CGAATGAGTGTGGATGACAATAGGGAGGTCAGTTCTCATAACTTGTACGTTTCGT
CMB109	GCCGGATCCGGGCCTCATGTTCGAATGAGTGTGGATGACAATAGG
CMB112	CACTATAGGGAGCTCAGAATAAACGCTCAACAACGGATCACACGCAAGCGAA
CMB113	GCGACCCTTAGACCCGAAGGACATAGATTCGCTTGC GTGTGATCCGTTGTTG
CMB114	GCCGGATCCGGGCCTCATGTTCGAAAGCGACCCTTAGACCCGAAGGACATAGA
CMB116	AGTCTCTCCTAGAGGGTGACGCGTCACCCCGACTGAGCTCAGTAACTTGA
CMB117	GCCGGATCCGGGCCTCATGTTCGAAACAGAGTCTCTCCTAGAGGGTGACGCGT
CMB119	CGAACC ACTACAATATGATATTAGATCTCCAGATCGGATATATTAATTTGATCTTG
CMB120	GCCGGATCCGGGCCTCATGTTCGAACCACTACAATATGATATTAGATCT
CMB122	CTCGCAAAAAGACTTGGGGAAGGAGCGAAAAAGTGACAGTCTCAGGC
CMB123	GCCGGATCCGGGCCTCATGTTCGAAACTCGCAAAAAGACTTGGGGAAGGA
CMB125	TCGCTATTTGGGCGGTAGAGCTTACCCCTCTCCCCGAACTCAGTAACTTTGAG
CMB126	CACTATAGGGAGCTCAGAATAAACGCTCAAGTTACTGAGCTCAGTCGGGGT
CMB127	GCCGGATCCGGGCCTCATGCTGAGCTCAGTAACTT GAGCGTTTATTCTGAGCTCCCTAT
CMB130	CACTATAGGGAGCTCAGAATAAACGCTCAAACCGATGAGCTCAGTCGGGGT
CMB131	AGTCTCTCCTAGAGGGTGACGCGTCACCCCGACTGAGCTCATCGGTTTGA
CMB132	GCCGGATCCGGGCCTCATGTTCGAAACAGAGTCTCTCCTAGAGGGTGACGCGT

CMB133	ATAGGGAGCTCAGAATAAACGCTCAAGATCAAATTTAATATATCCGATCTGGAGATCTAA
CMB134	CTATAGGGAGCTCAGAATAAACGCTCAACTGCCTGAGACTGCACTTTTTTCG
CMB139	CTCTCCCCGGAACATCGGTTTTGAGCGTTTATTCTGAGCTCCCTATAGTG
CMB140	TCGCTATTTGGGCGGTAGAGCTTACCCCTCTCCCCGGAACATCGGTTTTGAG
CMB141	GCCGGATCCGGGCCTCATGTGCGAATCGCTATTTGGGCGGTAGAGCTC
CMB142	CACTATAGGGAGCTCAGAATAAACGCTCAAAGTTACTGAGTTCCGGGGAGAG
CMB152	CTGGTCATGGCGGGCATTTAATTTCGGGCCTCATGTGCGAAACAGAG
CMB153	CTGGTCATGGCGGGCATTTAATTCTCGACCTCTGGGTATGTCGTT
CMB154	CTGGTCATGGCGGGCATTTAATTCCCATTTTCTCGCCCTCTTCG
CMB183	GCGGCTGTCTCCACAAGTCGCCGGATCCGGGCCT
SELEX Library template	CCGAAGCTTAATACGACTCACTATAGGGAGCTCAGAATAAACGCTCAA-[N ₅₀]- TTCGACATGAGGCCCGGATCCGGC
SELEX Forward Primer	CCGAAGCTTAATACGACTCACTATAGGGAGCTCAGAATAAACGCTCAA
SELEX Reverse Primer	GCCGGATCCGGGCCTCATGTGCGAA
Capture Oligo	Biotin-CTGGTCATGGCGGGCATTTAATTC